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(54) **Monoclonal-antibody-producing hybrid cell line, antibody and method of preparing it, therapeutic composition containing it and its diagnostic and therapeutic uses**

Einen monoklonalen Antikörper produzierende Hybridzelllinie, Antikörper und Verfahren zu seiner Herstellung, diesen Antikörper enthaltende therapeutische Zusammensetzung und deren diagnostische und therapeutische Verwendungen

Lignée cellulaire hybride produisant un anticorps monoclonal, anticorps et procédé pour sa préparation, composition thérapeutique le contenant et ses applications diagnostiques et thérapeutiques

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Remarks:

The file contains technical information submitted after the application was filed and not included in this specification

Description

Field of the invention

5 **[0001]** This invention relates generally to new hybrid cell lines and more specifically to hybrid cell lines for production of complement-fixing monoclonal antibody to an antigen found on all normal human T cells and cutaneous T lymphoma cells, to the antibody so produced, and to therapeutic and diagnostic compositions and diagnostic methods employing this antibody.

10 Description of the prior art

[0002] The fusion of mouse myeloma cells to spleen cells from immunized mice by Kohler and Milstein in 1975 [*Nature* 256, 495-497 (1975)] demonstrated for the first time that it was possible to obtain a continuous cell line making homogeneous (so-called "monoclonal") antibody. Since this seminal work, such effort has been directed to the pro-
15 duction of various hybrid cells (called "hybridomas") and to the use of the antibody made by these hybridomas for various scientific investigations. See, for example, *Current Topics in Microbiology and Immunology*, Volume 81-"Lymphocyte Hybridomas", F. Melchers, M. Potter, and N. Warner, Editors, Springer-Verlag, 1978, and references contained therein; C. J. Barnstable, *et al.*, *Cell*, 14, 9-20 (May, 1978); P. Parham and W. F. Bodmer, *Nature* 276, 397-399 (November, 1978); *Handbook of Experimental Immunology*, Third Edition, Volume 2, D. M. Wier, Editor, Blackwell, 1978,
20 Chapter 25; and *Chemical and Engineering News*, January 1, 1979, 15-17.

[0003] These references simultaneously indicate the rewards and complications of attempting to produce monoclonal antibody from hybridomas. While the general technique is well understood conceptually, there are many difficulties met and variations required for each specific case. In fact, there is no assurance, prior to attempting to prepare a given hybridoma, that the desired hybridoma will be obtained, that it will produce antibody if obtained, or that the antibody
25 so produced will have the desired specificity. The degree of success is influenced principally by the type of antigen employed and the selection technique used for isolating the desired hybridoma.

[0004] The attempted production of monoclonal antibody to human lymphocyte cell-surface antigens has been reported only in a few instances. See, for example, *Current Topics in Microbiology and Immunology*, *ibid*, 66-69 and 164-169. The antigens used in these reported experiments were cultured human lymphoblastoid leukemia and human
30 chronic lymphocytic leukemia cell lines. Many hybridomas obtained appeared to produce antibody to various antigens on all human cells. None of the hybridomas produced antibody against a predefined class of human lymphocytes.

[0005] It should be understood that there are two principal classes of lymphocytes involved in the immune system of humans and animals. The first of these (the thymus-derived cell or T cell) is differentiated in the thymus from haemopoietic stem cells. While within the thymus, the differentiating cells are termed "thymocytes." The mature T cells
35 emerge from the thymus and circulate between the tissues, lymphatics, and the bloodstream. These T cells form a large proportion of the pool of recirculating small lymphocytes. They have immunological specificity and are directly involved in cell-mediated immune responses (such as graft rejection) as effector cells. Although T cells do not secrete humoral antibodies, they are sometimes required for the secretion of these antibodies by the second class of lymphocytes discussed below. Some types of T cells play a regulating function in other aspects of the immune system.
40 The mechanism of this process of cell cooperation is not yet completely understood.

[0006] The second class of lymphocytes (the bone marrow-derived cells or B cells) are those which secrete antibody. They also develop from haemopoietic stem cells, but their differentiation is not determined by the thymus. In birds, they are differentiated in an organ analogous to the thymus, called the Bursa of Fabricius. In mammals, however, no equivalent organ has been discovered, and it is thought that these B cells differentiate within the bone marrow.

45 **[0007]** It is now recognized that T cells are divided into at least several subtypes, termed "helper", "suppressor", and "killer" T cells, which have the function of (respectively) promoting a reaction, suppressing a reaction, or killing (lysing) foreign cells. These subclasses are well understood for murine systems, but they have only recently been described for human systems. See, for example, R. L. Evans, *et al.*, *Journal of Experimental Medicine*, Volume 145, 221-232, 1977; and L. Chess and S. F. Schlossman-"Functional Analysis of Distinct Human T-Cell Subsets Bearing Unique
50 Differentiation Antigens", in *Contemporary Topics in Immunobiology*, O. Stutman, Editor, Plenum Press, 1977, Volume 7, 363-379.

[0008] The ability to identify or suppress classes or subclasses of T cells is important for diagnosis or treatment of various immunoregulatory disorders or conditions.

55 **[0009]** For example, certain leukemias and lymphomas have differing prognosis depending on whether they are of B cell or T cell origin. Thus, evaluation of the disease prognosis depends upon distinguishing between these two classes of lymphocytes. See, for example, A. C. Aisenberg and J. C. Long, *The American Journal of Medicine*, 58:300 (March, 1975); D. Belpomme, *et al.*, in *Immunological Diagnosis of Leukemias and Lymphomas*, S. Thierfelder, *et al.*, eds, Springer, Heidelberg, 1977, 33-45; and D. Belpomme, *et al.*, *British Journal of Haematology*, 1978, 38, 85. Certain

disease states (e.g., juvenile rheumatoid arthritis and certain leukemias) are associated with an imbalance of T cell subclasses. It has been suggested that autoimmune diseases generally are associated with an excess of "helper" T cells or a deficiency of certain "suppressor" T cells, while malignancies generally are associated with an excess of "suppressor" T cells. In certain leukemias, excess T cells are produced in an arrested stage of development. Diagnosis may thus depend on the ability to detect this imbalance or excess. See, for example, J. Kersey *et al.*, "Surface Markers Define Human Lymphoid Malignancies with Differing Prognoses" in *Haematology and Blood Transfusion*, Volume 20, Springer-Verlag, 1977, 17-24, and references contained therein.

[0010] On the therapeutic side, there is some suggestion, as yet not definitely proven, that administration of antibodies against the subtype of T cell in excess may have therapeutic benefit in autoimmune disease or malignancies. Antisera against the entire class of human T cells (so-called antihuman thymocyte globulin or ATG) has been reported useful therapeutically in patients receiving organ transplants. Since the cell-mediated immune response (the mechanism whereby transplants are rejected) depends upon T cells, administration of antibody to T cells prevents or retards this rejection process. See, for example, Cosimi, *et al.*, "Randomized Clinical Trial of ATG in Cadaver Renal Allograft Recipients: Importance of T Cell Monitoring", *Surgery* 40: 155-163 (1976) and references contained therein.

[0011] The identification and suppression of human T cell classes and subclasses has previously been accomplished by the use of spontaneous autoantibodies or selective antisera for human T cells obtained by immunizing animals with human T cells, bleeding the animals to obtain serum, and adsorbing the antiserum with (for example) autologous but not allogeneic B cells to remove antibodies with unwanted reactivities. The preparation of these antisera is extremely difficult, particularly in the adsorption and purification steps. Even the adsorbed and purified antisera contain many impurities in addition to the desired antibody, for several reasons. First, the serum contains millions of antibody molecules even before the T cell immunization. Second, the immunization causes production of antibodies against a variety of antigens found on all human T cells injected. There is no selective production of antibody against a single antigen. Third, the titer of specific antibody obtained by such methods is usually quite low, (e.g., inactive at dilutions greater than 1:100) and the ratio of specific to non-specific antibody is less than $1/10^6$.

[0012] See, for example, the Chess and Schlossman article referred to above (at pages 365 and following) and the Chemical and Engineering News article referred to above, where the deficiencies of prior art antisera and the advantages of monoclonal antibody are described.

Summary of the invention

[0013] There has now been discovered a novel hybridoma (designated OKT3) which is capable of producing novel complement-fixing monoclonal antibody against an antigen found on essentially all normal human peripheral T cells and cutaneous T lymphoma cells. The antibody so produced is mono-specific for a single determinant on normal human T cells and cutaneous T lymphoma cells and contains essentially no other anti-human immunoglobulin, in contrast to prior art antisera (which are inherently contaminated with antibody reactive to numerous human antigens) and to prior art monoclonal antibodies (which are not monospecific for a human T cell antigen). Moreover, this hybridoma can be cultured to produce antibody without the necessity of immunizing and killing animals, followed by the tedious adsorption and purification steps necessary to obtain even the impure antisera of the prior art.

[0014] It is accordingly one object of this invention to provide hybridomas which produce antibodies against an antigen found on essentially all normal human T cells and cutaneous T lymphoma cells.

[0015] It is a further aspect of the present invention to provide methods for preparing these hybridomas.

[0016] A further object of the invention is to provide essentially homogeneous antibody against an antigen found on essentially all normal human T cells and cutaneous T lymphoma cells.

[0017] A still further object is to provide compositions for treatment or diagnosis and methods of diagnosis of disease employing these antibodies.

[0018] Other objects and advantages of the invention will become apparent from the examination of the present disclosure.

[0019] In satisfaction of the foregoing objects and advantages, there is provided by this invention a novel hybridoma producing novel antibody to an antigen found on essentially all normal human T cells and cutaneous T lymphoma cells, the antibody itself, and diagnostic and therapeutic compositions employing the antibody. The hybridoma was prepared generally following the method of Milstein and Kohler. Following immunization of mice with normal E rosette positive human T cells, the spleen cells of the immunized mice were fused with cells from a mouse myeloma line and the resultant hybridomas screened for those with supernatants containing antibody which gave selective binding to normal E rosette positive human T cells. The desired hybridomas were subsequently cloned and characterised. As a result, a hybridoma was obtained which produces antibody (designated OKT3) against an antigen on essentially all normal human T cells. Not only does this antibody react with essentially all normal human peripheral T cells, but it also does not react with other normal peripheral blood lymphoid cells.

[0020] In view of the difficulties indicated in the prior art and the lack of success reported using malignant cell lines

as the antigen, it was surprising that the present method provided the desired hybridoma. It should be emphasized that the unpredictable nature of hybrid cell preparation does not allow one to extrapolate from one antigen or cell system to another. In fact, the present applicants have discovered that using a T cell malignant cell line as the antigen caused formation of hybridomas which did not produce the desired antibody. Attempts to use purified antigens separated from the cell surfaces were also unsuccessful.

[0021] Both the subject hybridoma and the antibody produced, thereby are identified herein by the designation "OKT3", the particular material referred to being apparent from the context.

[0022] A sample of the subject hybridoma OKT3 was deposited at the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD, 20852, United States of America, on April 26, 1979, and has been assigned the ATCC number CRL 8001.

[0023] The present invention provides mouse complement-fixing monoclonal antibody which (i) reacts with essentially all normal human peripheral T-cells but (ii) does not react with any of the normal human peripheral cells in the group comprising B cells, null cells and macrophages.

[0024] Preferably the antibody is of class IgG, most preferably of class IgG₂. The antibody reacts with essentially all cutaneous T lymphoma cells.

[0025] The antibody may be produced by a hybridoma formed by a fusion of spleen cells from a mouse previously immunised with normal E-rosette positive purified human T cells and cells from a mouse myeloma line. The monoclonal antibody is preferably produced from hybridoma ATCC CRL 8001 (OKT3).

[0026] According to another aspect of the present invention there is provided a hybridoma, which produces an antibody according to the invention, formed by fusion of spleen cells from a mouse previously immunised with normal E-rosette positive purified human T cells and cells from a mouse myeloma line.

[0027] The hybridoma is preferably ATCC CRL 8001 (OKT3).

[0028] According to yet another aspect of the present invention, there is provided a method for preparing an antibody according to the invention, which comprises the steps of:

- (i) immunizing mice with normal E rosette positive purified human T cells;
- (ii) removing the spleens from said mice and making a suspension of spleen cells;
- (iii) fusing said spleen cells with mouse myeloma cells in the presence of a fusion promoter;
- (iv) diluting and culturing the fused cells in separate wells in a medium which will not support the unfused myeloma cells;
- (v) evaluating the supernatant in each well containing a hybridoma for the presence of an antibody having any of the properties specified above;
- (vi) selecting and cloning hybridomas producing the desired antibody; and
- (vii) recovering the antibody from the supernatant above said clones.

[0029] Alternatively the method comprises the steps of:

- (i) immunizing mice with normal E rosette positive purified human T cells;
- (ii) removing the spleens from said mice and making a suspension of the spleen cells;
- (iii) fusing said spleen cells with mouse myeloma cells in the presence of a fusion promoter;
- (iv) diluting and culturing the fused cells in separate wells in a medium which will not support the unfused myeloma cells;
- (v) evaluating the supernatant in each well containing a hybridoma for presence of an antibody having any of the properties specified above;
- (vi) selecting and cloning hybridomas producing the desired antibody;
- (vii) transferring said clones intraperitoneally into mice; and
- (viii) harvesting the malignant ascites or serum from said mice.

[0030] According to a further aspect of the present invention a method of preparing an antibody comprises culturing the hybridoma ATCC CRL 8001 in a suitable medium and recovering the antibody from the supernatant above said hybridoma.

[0031] Alternatively the method comprises injecting into a mouse the hybridoma ATCC CRL 8001 and recovering the antibody from the malignant ascites or serum of said mouse.

[0032] The preparation and characterization of the hybridoma and the resultant antibody will be better understood by reference to the following description and Examples.

Detailed description of the invention

[0033] The method of preparing the hybridoma generally comprises the following steps:

5 A. Immunizing mice with E rosette positive purified normal human peripheral T cells. While it has been found that female CAF₁ mice (a first generation hybrid between Balb/cJ and A/J mice) are preferred, it is contemplated that other mouse strains could be used. The immunization schedule and T cell concentration should be such as to produce useful quantities of suitably primed splenocytes. Three immunizations at fourteen day intervals with 2×10^7 cells/mouse/injection in 0.2 ml phosphate buffered saline has been found to be effective.

10 B. Removing the spleens from the immunized mice and making a spleen suspension in an appropriate medium. About one ml of medium per spleen is sufficient. These experimental techniques are well-known.

C. Fusing the suspended spleen cells with mouse myeloma cells from a suitable cell line by the use of a suitable fusion promoter. The preferred ratio is about 5 spleen cells per myeloma cell. A total volume of about 0.5-1.0 ml of fusion medium is appropriate for about 10^8 splenocytes. Many mouse myeloma cell lines are known and available, generally from members of the academic community or various deposit banks, such as the Salk Institute Cell Distribution Center, La Jolla, CA. The cell line used should preferably be of the so-called "drug resistant" type, so that unfused myeloma cells will not survive in a selective medium, while hybrids will survive. The most common class is 8-azaguanine resistant cell lines, which lack the enzyme hypoxanthine guanine phosphoribosyl transferase and hence will not be supported by HAT (hypoxanthine, aminopterin, and thymidine) medium. It is also generally preferred that the myeloma cell line used be of the so-called "non-secreting" type, in that it does not itself produce any antibody, although secreting types may be used. In certain cases, however, secreting myeloma lines may be preferred. While the preferred fusion promoter is polyethylene glycol having an average molecular weight from about 1000 to about 4000 (commercially available as PEG 1000, etc.), other fusion promoters known in the art may be employed.

20 D. Diluting the culturing in separate containers, the mixture of unfused spleen cells, unfused myeloma cells, and fused cells in a selective medium which will not support the unfused myeloma cells for a time sufficient to allow death of the unfused cells (about one week). The dilution may be a type of limiting one, in which the volume of diluent is statistically calculated to isolate a certain number of cells (e.g., 1-4) in each separate container (e.g., each well of a microtiter plate). The medium is one (e.g., HAT medium) which will not support the drug-resistant (e.g., 8-azaguanine resistant) unfused myeloma cell line. Hence, these myeloma cells perish. Since the unfused spleen cells are non-malignant, they have only a finite number of generations. Thus, after a certain period of time (about one week) these unfused spleen cells fail to reproduce. The fused cells, on the other hand, continue to reproduce because they possess the malignant quality of the myeloma parent and the ability to survive in the selective medium of the spleen cell parent.

30 E. Evaluating the supernatant in each container (well) containing a hybridoma for the presence of antibody to E rosette positive purified human T cells.

F. Selecting (e.g., by limiting dilution) and cloning hybridomas producing the desired antibody.

40 [0034] Once the desired hybridoma has been selected and cloned, the resultant antibody may be produced in one of two ways. The purest monoclonal antibody is produced by *in vitro* culturing of the desired hybridoma in a suitable medium for a suitable length of time, followed by recovery of the desired antibody from the supernatant. The suitable medium and suitable length of culturing time are known or are readily determined. This *in vitro* technique produces essentially monospecific monoclonal antibody, essentially free from other specific antihuman immuno globulin. There is a small amount of other immuno globulin present since the medium contains xenogeneic serum (e.g., fetal calf serum). However, this *in vitro* method may not produce a sufficient quantity or concentration of antibody for some purposes, since the concentration of monoclonal antibody is only about 50 µg/ml.

45 [0035] To produce a much greater concentration of slightly less pure monoclonal antibody, the desired hybridoma may be injected into mice, preferably syngenic or semisyngenic mice. The hybridoma will cause formation of antibody-producing tumors after a suitable incubation time, which will result in a high concentration of the desired antibody (about 5-20 mg/ml) in the bloodstream and peritoneal exudate (ascites) of the host mouse. Although these host mice also have normal antibodies in their blood and ascites, the concentration of these normal antibodies is only about 5% of the monoclonal antibody concentration. Moreover, since these normal antibodies are not antihuman in their specificity, the monoclonal antibody obtained from the harvested ascites or from the serum is essentially free of any contaminating antihuman immunoglobulin. This monoclonal antibody is high titer (active at dilutions of 1:100,000 or higher) and high ratio of specific to non-specific immunoglobulin (about 1/20). Immunoglobulin produced incorporating the κ light myeloma chains are non-specific, "nonsense" peptides which merely dilute the monoclonal antibody without detracting from its specificity.

Example I

Production of monoclonal antibodies

A. Immunization and somatic cell hybridization

[0036] Femal CAF₁ mice (Jackson Laboratories; 6-8 weeks old) were immunized intraperitoneally with 2×10⁷ E rosette purified T cells in 0.2 ml of phosphate buffered saline at 14-day intervals. Four days after the third immunization, spleens were removed from the mice, and a single cell suspension was made by pressing the tissue through a stainless steel mesh.

[0037] Cell fusion was carried out according to the procedure developed by Kohler and Milstein. 1×10⁸ splenocytes were fused in 0.5 ml of a fusion medium comprising 35% polyethylene glycol (PEG 1000) and 5% dimethylsulfoxide in RPMI 1640 medium (Gibco, Grand Island, NY) with 2×10⁷ P3X63Ag8U1 myeloma cells supplied by Dr. M. Scharff, Albert Einstein College of Medicine, Bronx, NY. These myeloma cells secrete IgG₁ κ light chains.

B. Selection and growth of hybridoma

[0038] After cell fusion, cells were cultured in HAT medium (hypoxanthine, aminopterin, and thymidine) at 37°C with 5% CO₂ in a humid atmosphere. Several weeks later, 40 to 100 μl of supernatant from cultures containing hybridomas were added to a pellet of 10⁶ peripheral lymphocytes separated into E rosette positive (E⁺) and E rosette negative (E⁻) populations, which were prepared from blood of healthy human donors as described by Mendes (*J. Immunol.* 111: 860, 1973). Detection of mouse hybridoma antibodies binding to these cells was determined by radioimmunoassay and/or indirect immunofluorescence. In the first method, the cells were initially reacted with 100 μl of affinity-purified ¹²⁵I goat-anti-mouse IgG (10⁶ cpm/μg; 500 μg/μl). (Details of iodination of goat-anti-mouse IgG were described by Kung, et al., *J. Biol. Chem.* 251(8): 2399, 1976). Alternatively, cells incubated with culture supernatants were stained with a fluoresceinated goat-anti-mouse IgG (G/M FITC) (Meloy Laboratories, Springfield, VA; F/p=2.5) and the fluorescent antibody-coated cells were subsequently analyzed on the Cytofluorograf FC200/4800A (Ortho Instruments, Westwood, MA) as described in Example III. Hybridoma cultures containing antibodies reacting specifically with E⁺ lymphocytes (T cells) were selected and cloned. Subsequently, the clones were transferred intraperitoneally by injecting 1×10⁷ cells of a given clone (0.2 ml volume) into CAF₁ mice primed with 2,6,10,14-tetramethylpentadecane, sold by Aldrich Chemical Company under the name Pristine. The malignant ascites from these mice were then used to characterize lymphocytes as described below in Example II. The subject hybrid antibody OKT3 was demonstrated by standard techniques to be of IgG₂ subclass and to fix complement.

Example II

Characterization of OKT3 reactivity

[0039]

A. Isolation of lymphocyte populations

Human peripheral blood mononuclear cells were isolated from healthy volunteer donors (ages 15-40) by Ficoll-Hypaque density gradient centrifugation (Pharmacia Fine Chemicals, Piscataway, NJ) following the technique of Boyum, *Scand. J. Clin. Lab. Invest.* 21 (Suppl. 97): 77, 1968. Unfractionated mononuclear cells were separated into surface Ig⁺ (B) and Ig⁻ (Tplus Null) populations by Sephadex G-200 anti-F(ab')₂ column chromatography as previously described by Chess, et al., *J. Immunol.* 113: 1113 (1974). T cells were recovered by E rosetting the Ig⁻ population with 5% sheep erythrocytes (Microbiological Associates, Bethesda, MD). The rosetted mixture was layered over Ficoll-Hypaque and the recovered E⁺ pellet treated with 0.155M NH₄Cl (10 ml per 10⁸ cells). The T cell population so obtained was <2% EAC rosette positive and >95% E rosette positive as determined by standard methods. In addition, the non-rosetting Ig⁻ (Null cell) population was harvested from the Ficoll interface. This latter population was <5% E⁺ and ≤2% slg⁺. The surface Ig⁺ (B) population was obtained from the Sephadex G-200 column following elution with normal human gamma globulin as previously described. This population was >95% surface Ig⁺ and <5% E⁺.

Normal human macrophages were obtained from the mononuclear population by adherence to polystyrene. Thus, mononuclear cells were resuspended in final culture media (RPMI 1640, 2.5mM HEPES [4-(2-hydroxyethyl)-1-piperazinepropane sulfonic acid] buffer, 0.5% sodium bicarbonate, 200mM L-glutamine, and 1% penicillin-streptomycin, supplemented with 20% heat-inactivated human AB serum) at a concentration of 2×10⁶ cells and incubated in plastic petri dishes (100×20 mm) (Falcon Tissue Culture Dish; Falcon, Oxnard, CA) at 37°C overnight.

After extensive washing to remove non-adherent cells, the adherent population was detached by brisk washing with cold serum-free medium containing 2.5mM EDTA and occasional scraping with the rubber tip of a disposable syringe plunger. Greater than 85% of the cell population was capable of ingesting latex particles and had morphologic characteristics of monocytes by Wright-Giemsa staining.

B. Normal thymus

Normal human thymus gland was obtained from patients aged two months to 14 years undergoing corrective cardiac surgery. Freshly obtained portions of the thymus gland were immediately placed in 5% fetal calf serum in medium 199 (Gibco), finely minced with forceps and scissors, and subsequently made into single cell suspensions by being pressed through wire mesh. The cells were next layered over Ficoll-Hypaque and spun and washed as previously described in section A above. The thymocytes so obtained were >95% viable and ≥90% E rosette positive.

C. Cell lines

Epstein-Barr Virus (EBV) transformed B cell lines from four normal individuals (Laz 007, Laz 156, Laz 256, and SB) and T cell lines CEM, HJD-1, Laz 191, and HM1 established from leukemic patients were provided by Dr. H. Lazarus, Sidney Farber Cancer Institute, Boston, MA.

D. T Acute lymphoblastic leukemia (T-ALL) cells and T chronic lymphatic leukemia (T-CLL) cells

Leukemia cells were obtained from 12 patients with T-ALL. These individuals' cells had previously been determined to be of T cell lineage by their spontaneous rosette formation with sheep erythrocytes (>20% E') and reactivity with T cell specific hetero-antisera, anti-HTL (anti-B.K.) and A99, as previously described by Schlossman, *et al.*, *Proc. Nat. Acad. Sci.* 73: 1288 (1976). Tumor cells from three individuals were reactive (TH₂⁺) with rabbit and/or equine anti-TH₂ while cells from the remaining nine were non-reactive (TH₂⁻). Leukemic cells from two patients with TH₂⁻ T-CLL were also utilized. Both acute and chronic T cell leukemia cells were cryopreserved in -196°C vapor phase liquid nitrogen in 10% dimethylsulfoxide and 20% AB human serum until the time of surface characterization. The tumor populations analyzed were >90% blasts by Wright-Giemsa morphology in all instances.

Example III

Cytofluorographic analysis

[0040] Cytofluorographic analysis of all cell populations was performed by indirect immunofluorescence with fluorescein-conjugated goat-anti-mouse IgG (G/M FITC) (Meloy Laboratories) on a Cytofluorograf FC200/4800A (Ortho Instruments). In brief, $1-2 \times 10^6$ cells were treated with 0.15 ml OKT3 at a 1:1000 dilution, incubated at 4°C for 30 minutes, and washed twice. The cells were then reacted with 0.15 ml of a 1:40 dilution G/M FITC at 4°C for 30 minutes, centrifuged, and washed three times. These cells were then analyzed on the Cytofluorograf and the intensity of fluorescence per cell recorded on a pulse height analyzer. A similar pattern of reactivity was observed at a dilution of 1:100,000, but further dilution caused loss of reactivity. Background staining was obtained by substituting a 0.15 ml aliquot of 1:1000 ascites from a Balb/cJ mouse intraperitoneally immunized with a non-producing hybrid clone.

Brief description of the drawings

[0041] Figure 1 shows the fluorescence pattern obtained on the Cytofluorograf after reacting normal human peripheral T cells with OKT3 at a 1:1000 dilution and G/M FITC. For comparison, results with monoclonal antibodies OKT1 and OKT4 are shown under equivalent conditions in Figures 1-5.

[0042] Figure 2 shows the fluorescence pattern obtained on the Cytofluorograf after reacting human thymocytes with OKT3 and G/M FITC.

[0043] Figure 3 shows the fluorescence pattern obtained on the Cytofluorograf after reacting leukemic cells from B cell chronic lymphoblastic leukemia patients with OKT3 and G/M FITC.

[0044] Figure 4 shows the fluorescence pattern obtained on the Cytofluorograf after reacting the human T cell line HJD-1 with OKT3 and G/M FITC.

[0045] Figure 5 shows the fluorescence pattern obtained on the Cytofluorograf after reacting the human T cell line CEM with OKT3 and G/M FITC.

[0046] The data in Figures 1-5 plus additional data for OKT3 (as well as OKT1 and OKT4) are summarized in Table I.

[0047] The production of the hybridoma and the production and characterization of the resulting monoclonal antibody were conducted as described in the above Examples. Although large quantities of the subject antibody were prepared by injecting the subject hybridoma intraperitoneally into mice and harvesting the malignant ascites, it is clearly con-

templated that the hybridoma could be cultured *in vitro* by techniques well-known in the art and the antibody removed from the supernatant.

[0048] As shown in Figure 1, the entire human peripheral blood T cell population of a given normal individual is reactive with OKT3 the entire B cell, null cell, and macrophage populations isolated from the same individual are unreactive with OKT3. Similar results were obtained on populations of lymphocytes from fifteen other normal individuals. The monoclonal antibody is thus characterized in that it is reactive with an antigen contained on the surface of essentially all normal human peripheral T cells, while being unreactive with any antigens on the surface of the other three cell types discussed above. This differential reactivity is one test by which the subject antibody OKT3 may be detected and distinguished from other antibodies.

[0049] The subject antibody is also useful for determining the proportion of circulating lymphocytes that are T cells. As shown in Table 1, $\geq 95\%$ of all T cells react with OKT3 antibody. The present invention thus includes a method for determining the proportion of lymphocytes circulating in an individual that are T cells which comprises mixing OKT3 antibody with a sample of said lymphocytes obtained from the individual and determining the proportion of said sample of lymphocytes which are OKT3⁺, and thus T cells.

[0050] The specific reaction of OKT3 antibody with an antigen on cutaneous T cell lymphomas is illustrated by Table II, where the distinction from OKT1 and OKT4 is shown. The present antibody thus provides a reagent for confirming a diagnosis of cutaneous T cell lymphoma in a patient suspected of having said disease. It is contemplated that treatment of cutaneous T cell lymphoma can therefore be effected by administration of a therapeutically effective amount of OKT3 antibody.

[0051] According to the present invention there are provided a hybridoma capable of producing antibody against an antigen found on essentially all normal human T cells and cutaneous T lymphoma cells, a method for producing this hybridoma, monoclonal antibody against an antigen found on essentially all human T cells, methods for producing the antibody, and methods for diagnosis of disease employing this antibody.

[0052] Although only a single hybridoma producing a single monoclonal antibody against human T cell antigen is described, it is contemplated that the present invention encompasses all monoclonal antibodies exhibiting the characteristics described herein. It was determined that the subject antibody OKT3 belongs to the subclass IgG₂, which is one of four subclasses of murine IgG. These subclasses of immunoglobulin G differ from one another in the so-called "fixed" regions, although an antibody to a specific antigen will have a so-called "variable" region which is functionally identical regardless of which subclass of immunoglobulin G it belongs to. That is, a monoclonal antibody exhibiting the characteristic described herein may be of subclass IgG₁, IgG_{2a}, IgG_{2b}, or IgG₃, or of classes IgM, IgA, or other known Ig classes. The differences among these classes or subclasses will not affect the selectivity of the reaction pattern of the antibody, but may affect the further reaction of the antibody with other materials, such as (for example) complement or anti-mouse antibodies. Although the subject antibody is specifically IgG₂, it is contemplated that antibodies having the patterns of reactivity illustrated herein are included within the subject invention regardless of the immunoglobulin class or subclass to which they belong.

[0053] Further included within the subject invention are methods for preparing the monoclonal antibodies described above employing the hybridoma technique illustrated herein. Although only one example of a hybridoma is given herein, it is contemplated that one skilled in the art could follow the immunization, fusion, and selection methods provided herein and obtain other hybridomas capable of producing antibodies having the reactivity characteristics described herein. Since the individual hybridoma produced from a known mouse myeloma cell line and spleen cells from a known species of mouse cannot be further identified except by reference to the antibody produced by the hybridoma, it is contemplated that all hybridomas producing antibody having the reactivity characteristics described above are included within the subject invention, as are methods for making this antibody employing the hybridoma.

[0054] Further aspects of the invention are methods of diagnosis of disease employing the monoclonal antibody OKT3 or any other monoclonal antibody exhibiting the pattern of reactivity provided herein. As discussed above, the subject antibody allows treatment of patients having certain T cell chronic lymphoblastic leukemias by administration of a therapeutically-effective amount thereof. Administration of a therapeutically-effective amount of OKT3 antibody to an individual subject undergoing organ transplant will reduce or eliminate the rejection of this transplant. The subject antibody also allows detection of cutaneous T cell lymphoma in an individual by mixing a lymphoma T cell composition from said individual with a diagnostically-effective amount of OKT3 antibody. The presence of a reaction confirms the identity of the disease. The cutaneous T cell lymphoma may be treated by administering to an individual in need of such treatment a therapeutically-effective amount of OKT3 antibody. This antibody will react with and reduce the amount of T lymphoma cells, thus ameliorating the disease. The present invention additionally includes diagnostic and therapeutic compositions comprising (respectively) a diagnostically-effective or therapeutically-effective amount of OKT3 antibody in a diagnostically or pharmaceutically acceptable carrier.

[0055] Monoclonal antibodies OKT1 and OKT4 referred to herein are described in our copending European patent applications Nos. 80300829.1 and 80301356.4 respectively.

TABLE I

Monoclonal antibody reactivity and properties			
	Monoclonal antibodies		
	OKT1	OKT3	OKT4
% Reactivity with:			
Peripheral T-cells (10 samples)	>95%	>95%	95%
Peripheral B-cells (10 samples)	<2%	<2%	<2%
Peripheral Null cells (10 samples)	<2%	<2%	<2%
Thymocytes* (8 samples)	5-10%	5-10%	80%
Reactivity with:			
T-chronic lymphatic Leukemia (3 cases)	+	+(1);-(2)	-
T-acute lymphatic Leukemia (8 cases)	-	-	-
Null acute lymphatic Leukemia (15 cases)	-	-	-
B-chronic lymphatic Leukemia (6 cases)	+(4);-(2)	-	-
B-cell lines* (4)	-	-	-
T-cell lines* HJD-1	+	(±)	-
CEM	+	-	+
Laz 191	+	-	-
HM1	+	-	-
IgG Subclass	IgG ₁	IgG ₂	IgG ₂
Complement fixation	-	+	+

*From patients aged 2 months to 18 years.

*Obtained from Dr. H. Lazarus, Sidney Farber Cancer Center. B cell lines Laz 256, 156, 007 and SB obtained by Epstein-Barr virus transformation of human peripheral B cells and HJD-1, CEM, Laz 191, and HM1 established from leukemic patients.

TABLE II

Patient's name	Cutaneous T-cell lymphoma	Monoclonal Antibody assays		
	Diagnosis	OKT1	OKT3	OKT4
E. McBride	Sezary Blast Crisis; PBL	+	+	-
C. O. Okley	Mycosis Fungoides; Node	-	+	+
Odom	Mycosis Fungoides; Node	+	+	-
Montalbono	? Node	+	+	+
Source of cells: PBL=peripheral blood lymphocytes Node=lymph node				

Claims

1. Mouse complement-fixing monoclonal antibody which (i) reacts with essentially all normal human peripheral T-cells but (ii) does not react with any of the normal human peripheral cells in the group comprising B cells, null cells and macrophages.

2. Monoclonal antibody according to claim 1, of class IgG.
3. Monoclonal antibody according to claim 2, of class IgG₂.
- 5 4. Monoclonal antibody according to any one of claims 1 to 3, which reacts with essentially all cutaneous T lymphoma cells.
- 10 5. Monoclonal antibody according to any one of claims 1 to 4, which is produced by hybridoma formed by a fusion of spleen cells from a mouse previously immunised with normal E-rosette positive purified human T cells and cells from a mouse myeloma line.
6. Monoclonal antibody which is produced from hybridoma ATCC CRL 8001 (OKT3).
- 15 7. A therapeutic composition of matter comprising, in admixture with a pharmaceutically acceptable carrier, a therapeutically effective amount of the antibody of any one of claims 1 to 6, said amount being effective to reduce or eliminate the rejection of a transplant by an organ transplant recipient.
- 20 8. A hybridoma which produces a monoclonal antibody according to any one of claims 1 to 5, formed by fusion of spleen cells from a mouse previously immunised with normal E-rosette positive purified human T cells and cells from a mouse myeloma line.
9. Hybridoma ATCC CRL 8001 (OKT3).
- 25 10. Monoclonal antibody according to any one of claims 1 to 6 for use in the treatment of an organ transplant recipient to reduce or eliminate allograft rejection of said transplant organ.
- 30 11. A method for determining the proportion of lymphocytes circulating in an individual that are T cells which comprises mixing the monoclonal antibody of any one of claims 1 to 6 with a sample of said lymphocytes obtained from said individual and determining the proportion of said sample of lymphocytes which react with said antibody, and are thus T cells.
12. A method for preparing a monoclonal antibody according to any one of claims 1 to 5, which comprises the steps of:
 - 35 i) immunizing mice with normal E rosette positive purified human T cells;
 - ii) removing the spleens from said mice and making a suspension of spleen cells;
 - iii) fusing said spleen cells with mouse myeloma cells in the presence of a fusion promoter;
 - iv) diluting and culturing the fused cells in separate wells in a medium which will not support the unfused myeloma cells;
 - 40 v) evaluating the supernatant in each well containing a hybridoma for the presence of an antibody having the properties specified in any one of claims 1 to 5;
 - vi) selecting and cloning hybridomas producing the desired antibody; and
 - vii) recovering the antibody from the supernatant above said clones.
- 45 13. A method for preparing a monoclonal antibody according to any one of claims 1 to 5, which comprises the steps of:
 - i) immunizing mice with normal E rosette positive purified human T cells;
 - ii) removing the spleens from said mice and making a suspension of the spleen cells;
 - iii) fusing said spleen cells with mouse myeloma cells in the presence of a fusion promoter;
 - 50 iv) diluting and culturing the fused cells in separate wells in a medium which will not support the unfused myeloma cells;
 - v) evaluating the supernatant in each well containing a hybridoma for the presence of an antibody having the properties specified in any one of claims 1 to 5;
 - vi) selecting and cloning hybridomas producing the desired antibody;
 - vii) transferring said clones intraperitoneally into mice; and
 - 55 viii) harvesting the malignant ascites or serum from said mice.
14. A method for preparing a monoclonal antibody, which comprises culturing the hybridoma ATCC CRL 8001 in a suitable medium and recovering the antibody from the supernatant above said hybridoma.

15. A method for preparing a monoclonal antibody, which comprises injecting into a mouse the hybridoma ATCC CRL 8001 and recovering the antibody from the malignant ascites or serum of said mouse.
16. The antibody of claim 4 or claim 5 when dependent on claim 4, or claim 6 for use in confirming the presence of cutaneous T cell lymphoma in an individual.
17. The antibody of claim 4 or claim 5 when dependent on claim 4, or claim 6, for use in the treatment of cutaneous T cell lymphoma in an individual.
18. A method for preparing a therapeutic composition according to claim 7, comprising mixing an amount of an antibody according to any one of claims 1 to 6 effective to reduce or eliminate the rejection of a transplant by an organ transplant recipient, with a pharmaceutically acceptable carrier.

Patentansprüche

1. Monoklonaler Komplement-fixierender Maus-Antikörper, welcher (i) mit im wesentlichen allen normalen humanen peripheren T-Zellen reagiert, aber (ii) nicht mit irgendwelchen der normalen humanen peripheren Zellen in der B-Zellen, Null-Zellen und Macrophagen umfassenden Gruppe reagiert.
2. Monoklonaler Antikörper nach Anspruch 1 der Klasse IgG.
3. Monoklonaler Antikörper nach Anspruch 2 der Klasse IgG₂.
4. Monoklonaler Antikörper nach einem der Ansprüche 1 bis 3, welcher im wesentlichen mit allen kutanen T-Lymphom-Zellen reagiert.
5. Monoklonaler Antikörper nach einem der Ansprüche 1 bis 4, welcher durch ein Hybridom gebildet wird, das durch eine Fusion von Milzzellen einer Maus, die zuvor mit E-Rosette positiv gereinigten normalen humanen T-Zellen immunisiert worden ist, und Zellen aus einer Maus-Myelom-Linie ausgebildet ist.
6. Monoklonaler Antikörper, welcher von einem Hybridom ATCC CRL 8001 (OKT 3) gebildet wird.
7. Eine therapeutische Zusammensetzung, enthaltend, im Gemisch mit einem pharmazeutisch annehmbaren Träger, eine therapeutisch wirksame Menge des Antikörpers nach einem der Ansprüche 1 bis 6, welche Menge zur Verminderung oder Ausschaltung einer Transplantatabstoßung durch einen Organtransplantatempfänger wirksam ist.
8. Ein Hybridom, welches einen monoklonalen Antikörper nach einem der Ansprüche 1 bis 5 produziert, gebildet durch Fusion von Milzzellen einer zuvor mit E-Rosette positiv gereinigten normalen humanen T-Zellen immunisierten Maus und Zellen aus einer Maus-Myelom-Linie.
9. Das Hybridom ATCC CRL 8001 (OKT 3).
10. Monoklonaler Antikörper nach einem der Ansprüche 1 bis 6, zur Anwendung in der Behandlung eines Organtransplantatempfängers zur Verminderung oder Ausschaltung von Allograft-Abstoßung des genannten transplantierten Organs.
11. Ein Verfahren zur Bestimmung des Anteiles an in einem Individuum zirkulierenden Lymphocyten, die T-Zellen sind, welches ein Vermischen des monoklonalen Antikörpers nach einem der Ansprüche 1 bis 6 mit einer von dem genannten Individuum erhaltenen Probe der genannten Lymphocyten und ein Bestimmen des Anteiles der genannten Lymphocyten-Probe umfaßt, welche mit dem genannten Antikörper reagieren und somit T-Zellen sind.
12. Verfahren zur Herstellung eines monoklonalen Antikörpers gemäß einem der Ansprüche 1 bis 5, umfassend die Stufen:
 - i) Immunisieren von Mäusen mit E-Rosette-positiv gereinigten normalen humanen T-Zellen;
 - ii) Entfernen der Milz aus den genannten Mäusen und Bereiten einer Suspension von Milzzellen;

iii) Fusion der genannten Milzzellen mit Maus-Myelom-Zellen in Gegenwart eines Fusionspromotors;

iv) Verdünnen und Vermehren der vereinigten Zellen in getrennten Vertiefungen in einem Medium, welches die nicht-vereinigten Myelomzellen nicht erhält;

v) Auswerten des Überstandes in jeder Vertiefung, die ein Hybridom enthält, hinsichtlich des Vorliegens eines Antikörpers mit den in einem der Ansprüche 1 bis 5 angegebenen Eigenschaften;

vi) Auswählen und Klonen von Hybridomen, welche den gewünschten Antikörper bilden; und

vii) Gewinnen des Antikörpers aus dem Überstand über den genannten Klonen.

13. Ein Verfahren zur Herstellung eines monoklonalen Antikörpers nach einem der Ansprüche 1 bis 5, umfassend die Stufen:

i) Immunisieren von Mäusen mit E-Rosette-positiv gereinigten normalen humanen T-Zellen;

ii) Entfernen der Milz aus den genannten Mäusen und Bereiten einer Suspension von Milzzellen;

iii) Fusion der genannten Milzzellen mit Maus-Myelom-Zellen in Gegenwart eines Fusionspromotors;

iv) Verdünnen und Vermehren der vereinigten Zellen in getrennten Vertiefungen in einem Medium, welches die nicht-vereinigten Myelomzellen nicht erhält;

v) Auswerten des Überstandes in jeder Vertiefung, die ein Hybridom enthält, hinsichtlich des Vorliegens eines Antikörpers mit den in einem der Ansprüche 1 bis 5 angegebenen Eigenschaften;

vi) Auswählen und Klonen von Hybridomen, welche den gewünschten Antikörper bilden;

vii) Überführen der genannten Klone auf intraperitonealem Wege in Mäuse; und

viii) Ernten der malignen Asciten oder von Serum aus den genannten Mäusen.

14. Verfahren zur Herstellung eines monoklonalen Antikörpers, welches ein Vermehren des Hybridoms ATCC CRL 8001 in einem geeigneten Medium und das Gewinnen des Antikörpers aus dem Überstand über dem genannten Hybridom umfaßt.

15. Verfahren zur Herstellung eines monoklonalen Antikörpers, welches ein Injizieren des Hybridoms ATCC CRL 8001 in eine Maus und das Gewinnen des Antikörpers aus den malignen Asciten oder dem Serum dieser Maus umfaßt.

16. Der Antikörper nach Anspruch 4 oder Anspruch 5, wenn abhängig von Anspruch 4, oder nach Anspruch 6, zur Anwendung in der Bestätigung des Vorliegens von kutanem T-Zellen-Lymphom in einem Individuum.

17. Der Antikörper nach Anspruch 4 oder Anspruch 5, wenn abhängig von Anspruch 4, oder nach Anspruch 6, zur Anwendung in der Behandlung von kutanem T-Zellen-Lymphom in einem Individuum.

18. Verfahren zur Herstellung einer therapeutischen Zusammensetzung gemäß Anspruch 7, umfassend das Vermischen einer zur Verminderung oder Beseitigung einer Transplantatabstoßung durch einen Organtransplantatempfänger wirksamen Menge eines Antikörpers gemäß einem der Ansprüche 1 bis 6 mit einem pharmazeutisch annehmbaren Träger.

Revendications

1. Anticorps monoclonal fixant le complément de souris qui (i) réagit avec essentiellement toutes les cellules T périphériques humaines mais (ii) ne réagit avec aucune des cellules périphériques humaines normales dans le groupe comprenant les cellules B, les cellules null et les macrophages.

2. Anticorps monoclonal selon la revendication 1, de classe Ig G.
3. Anticorps monoclonal selon la revendication 2, de classe Ig G₂.
- 5 4. Anticorps monoclonal selon l'une quelconque des revendications 1 à 3, qui réagit avec essentiellement toutes les cellules de lymphome T cutanées.
5. Anticorps monoclonal selon l'une quelconque des revendications 1 à 4, qui est produit par un hybridome formé par une fusion de cellules de rate provenant d'une souris préalablement immunisée avec des cellules T humaines normales purifiées positives à la rosette E et des cellules provenant d'une lignée de myélome de souris.
- 10 6. Anticorps monoclonal qui est produit à partir de l'hybridome ATCC CRL 8001 (OKT3).
- 15 7. Composition thérapeutique comprenant, mélangée à un support pharmaceutiquement acceptable, une quantité thérapeutiquement efficace de l'anticorps de l'une quelconque des revendications 1 à 6, ladite quantité étant efficace pour réduire ou éliminer le rejet d'une greffe par un récepteur de greffe d'organe.
- 20 8. Hybridome produisant un anticorps monoclonal selon l'une quelconque des revendications 1 à 5, formé par fusion de cellules de rate provenant d'une souris préalablement immunisée avec des cellules T humaines normales purifiées positives à la rosette E et des cellules provenant d'une lignée de myélome de souris.
9. L'hybridome ATCC CRL 8001 (OKT3).
- 25 10. Anticorps monoclonal selon l'une quelconque des revendications 1 à 6 pour application dans le traitement d'un récepteur de greffe d'organe pour réduire ou éliminer le rejet d'une allogreffe dudit organe greffé.
- 30 11. Procédé pour déterminer la proportion de lymphocytes circulants chez un individu qui sont des cellules T, dans lequel on mélange l'anticorps monoclonal de l'une quelconque des revendications 1 à 6 avec un échantillon desdits lymphocytes obtenu à partir dudit individu et on détermine la proportion dudit échantillon de lymphocytes qui réagissent avec ledit anticorps, et sont ainsi des cellules T.
- 35 12. Procédé pour préparer un anticorps monoclonal selon l'une quelconque des revendications 1 à 5, comprenant les étapes consistant à :
 - i) immuniser des souris avec des cellules T humaines normales purifiées positives à la rosette E ;
 - ii) enlever la rate des dites souris et faire une suspension de cellules de rate;
 - iii) fusionner lesdites cellules de rate avec des cellules de myélome de souris en présence d'un promoteur de fusion;
 - 40 iv) diluer et cultiver les cellules fusionnées dans des réservoirs séparés dans un milieu qui n'entretient pas les cellules de myélome non fusionnées;
 - v) évaluer dans le surnageant de chaque réservoir contenant un hybridome la présence d'un anticorps ayant les propriétés spécifiées dans l'une quelconque des revendications 1 à 5 ;
 - vi) sélectionner et cloner des hybridomes produisant l'anticorps désiré; et
 - 45 vii) recueillir l'anticorps à partir du surnageant au-dessus desdits clones.
- 50 13. Procédé pour préparer un anticorps monoclonal selon l'une quelconque des revendications 1 à 5, comprenant les étapes consistant à :
 - i) immuniser des souris avec des cellules T humaines normales purifiées positives à la rosette E ;
 - ii) enlever la rate desdites souris et faire une suspension des cellules de rate;
 - iii) fusionner lesdites cellules de rate avec des cellules de myélome de souris en présence d'un promoteur de fusion;
 - iv) diluer et cultiver les cellules fusionnées dans des réservoirs séparés dans un milieu qui n'entretient pas les cellules de myélome non fusionnées;
 - 55 v) évaluer dans le surnageant contenant un hybridome la présence d'un anticorps ayant les propriétés spécifiées dans l'une quelconque des revendications 1 à 5 ;
 - vi) sélectionner et cloner les hybridomes produisant l'anticorps désiré;
 - vii) transférer lesdits clones par voie intrapéritonéale à des souris; et

viii) recueillir l'ascite ou sérum malin à partir desdites souris.

14. Procédé pour préparer un anticorps monoclonal, dans lequel on cultive l'hybridome ATCC CRL 8001 dans un milieu approprié et on recueille l'anticorps à partir du surnageant au-dessus dudit hybridome.

15. Procédé pour préparer un anticorps monoclonal, dans lequel on injecte à une souris l'hybridome ATCC CRL 8001 et on recueille l'anticorps à partir de l'ascite ou sérum malin de ladite souris.

16. Anticorps des revendications 4 ou 5 lorsqu'il dépend de la revendication 4, ou de la revendication 6 aux fins d'application pour confirmer la présence de lymphome de cellules T cutané chez un individu.

17. Anticorps des revendications 4 ou 5 lorsqu'il dépend de la revendication 4, ou de la revendication 6, aux fins d'application dans le traitement du lymphome des cellules T cutanées chez un individu.

18. Procédé pour préparer une composition thérapeutique selon la revendication 7, dans lequel on mélange une quantité d'un anticorps selon l'une quelconque des revendications 1 à 6 efficace pour réduire ou éliminer le rejet d'une greffe par un récepteur de greffe d'organe, avec un support pharmaceutiquement acceptable.

